International Journal of Bio-Technology and Research (IJBTR)
ISSN(P):2249-6858; ISSN(E):2249-796X
Vol. 6, Issue 6, Dec 2016, 17-26

TRANS STELLAR
•Journal Publications •Research Consultancy

STUDIES ON LEPTIN AND MC4R GENE IN OBESE POPULATION OF SOUTH WESTERN PROVINCE OF SAUDI ARABIA

MOHAMMED RIZWAN[1], AYESHA ALVI[2], RASHAD AL SUNOSI[3], MOHAMMED YAHYA AREESHI[4], SIDDIG IBRAHIM ABDEL WAHAB[5], ZAKI MUNAWWAR[6]& GOWHER NABI[7]

[1]*Department of Biochemistry, College of Nursing and Allied Health Sciences, Jazan University, Jazan, KSA*

[2]*Department of Genetics and Molecular Biology, College of Applied Medical Sciences, Jazan University, Jazan, KSA*

[3,5]*Substance Abuse Research Centre, Jazan University, Jazan KSA*

[4]*Department of Microbiology, College of Nursing and Allied Health Sciences, Jazan University, Jazan, KSA*

[6]*Central laboratory King Fahd Hospital,Abu Areesh, Jazan KSA*

[7]*Srinagar, Jammu and Kashmir, India*

ABSTRACT

Obesity has appeared as one of the major public health problem and economic burden worldwide. The rates of obesity are exploding in Saudi Arabia, approximately 30-50% of adult population is obese or overweight with an alarming increase of over 1% annually, one of the highest in the world. Sedentary life style, and complex polygenic factors are thought to play an important role. Studies pertaining to different physical factor responsible for this rise have been widely documented but biochemical and molecular aspects are not studied for this population. This study evaluated the serum leptin levels among Saudi obese, non-obese subjects. Concurrently, measuring the leptin resistance and possible major addition or deletions in MC4R gene using PCR. A total of 160 Saudi obese and non-obese subjects were analyzed for circulating leptin levels, MC4R gene polymorphism, leptin resistance and dyslipidemic parameters. It was found that the mean circulating levels of leptin were 14.41ng/ml in the obese subjects with a significant prevalence of leptin resistance (36.53%). Moreover the prevalence of dyslipidemia among them was estimated as 35.63%.

With regards to MC4R gene, 63% shown amplification with the first set of primer (Figure A) while 20% of them were amplified with second set of primer. However, in the remaining17% we didn't get any amplification indicating the possibility of mutation in the gene. Thus our study suggest significant prevalence of leptin resistance and possible mutations in melanocortin 4 receptor gene among the obese Saudi population however further whole genome sequencing and variance analysis from extremely obese subjects may yield important insights into genetic aspects of obesity in this population.

KEYWORDS: Obesity, Melanocortin 4 Receptor, Circulating Serum Leptin, Dyslipidemia

Received: Nov 10, 2016; **Accepted:** Nov 30, 2016; **Published:** Dec 17, 2016; **Paper Id.:** IJBTRDEC20163

Original Article

INTRODUCTION

Obesity is a polygenic multifactorial disorder, which occurs due to complex interplay of genetic, environmental and neurologic factors. It is described as the preventable cause of death by WHO, and considered as one of the most serious health problem of the 21st century. Obesity is a major risk factor for premature mortality fromcardiovascular diseases, dyslipidemia, degenerative joint disease, metabolic and various other diseases (Kopelman PG 2000). Therefore, evidence that its prevalence has been increasing and continues to increase in

most developed and developing countries is a cause for major concern (Barness LA 2007, Seidell JC, 1997 Prentice AM 2006).

The central and key players in obesity are melanocortinergic pathways which play an important role in mammalian energy homeostasis(Baskin DGI et al. 1999, Mizuno TM et al. 1999) and have functions of inhibiting food intake. Amongmelanocortinergic pathwaysleptin and melanocortin are considered as one of the major players.

Leptin is a protein hormone which is synthesized by the adipocytes and involved in energy homeostatis axis. Evidences suggest that the hypothalamic melanocortin system is directly influenced by leptin to regulate food intake andbody weight (Hwa et al. 2001) and it has been effectively demonstrated that serum leptin concentrations are highlycorrelated with body fat content. Moreover, the production of leptin is evidently related with gender, age and ethinicity.Most obese subjects have been shown to be insensitive to endogenous leptin production and have leptin resistance (ConsidineRV et al 1996,DyckDJ et al 2006). For example, absence of leptin is associated with massive obesity in ob^{-}/ob^{-}mice (Montague et al 1997).

Concurrently Melanocartin C4 receptor (MC4R) is a seven-transmembrane G-protein coupled receptor which is principally expressed in the brain, including the hypothalamus (ConsidineRV et al 1996,DyckDJ et al 2006). The importance of the MC4R in the regulation of human body weight became apparent in 1998 when mutations in the human MC4R gene were first described as animportant cause of obesity. Yeo et aland Vaisse et alreported heterozygous frame shift mutations in the human MC4R that co-segregated in a dominant fashion with severe early-onset obesity. Subsequently, multiple different missense and nonsense mutations in MC4R have also been reported, largely in subjects with severe obesity commencing in childhood.Moreover, the direct evidence that the MC4R is a key regulator of appetite and body weight was provided by Huszar and colleagues 1997, who demonstrated that mice with a targeted disruption of the MC4R gene have increased food intake, obesity and hyperinsulinaemia. Whereas heterozygous for the null allele showed an intermediate obesity phenotype when compared with their homozygous and wild-type littermates. Given the research work carried out, MC4R mutations now represent the commonest known monogenic cause of non-syndromic human obesity.

Saudi Arabia has been witnessing a sharp rise in the obese population national survey has put it at overall 9.3%. However other studies have put it as high as 33.9% in Hail to 11.9% in Jizan.There are many studies pertaining to prevalence of obesity among Saudi population, and physical factors such as sedentary life style and poor eating habits have been attributed as causes of obesity. However no biochemical,molecular and genetic factors have been so far reported for this population. In this backdrop this study aims at finding out the possible biochemicaland molecular factors for obesity within the framework of leptin levels, leptin resistance, MC4R gene, hyperlipidemia etc inobese and non-obese Saudi population.

MATERIALS AND METHOD

Enrollment of the Subjects

For the present study, a total 324 subjects in the age group of 18-25 years in accordance with the criteria given by WHO/ declaration of Helsinki (11) were randomly enrolled in the study. Their height and weight were taken with standardized methods (Seidell et al 1997) and BMI [weight (kg)/height (m2)] was calculated. Subjects with BMI 18.5 to <23 were considered as normal and the subjects with BMI ≥ 25 were considered as obese. Subjects less than 18.5 were

considered as underweight (Lean) and were excluded from the study. The exclusion criteria for the subjects were age below 18 years, prolonged fasting, taking lipid lowering medication, khat chewing or addicted to any other drug, pregnancy/lactation, and diabetics taking insulin or any other chronic disease. Inclusion criteria for the subjects was aged above 18 years, Non khat chewing, Non diabetic.

A standard questionnaire was given to the all participant to ascertain their eating habits and other necessary parameters such as anthropometric parameter (height, weight and waist circumference), hip circumference to calculate their waist to hip ratio, as per WHO recommendations. After ensuring overnight fasting for 12 hours, 3 ml of venous blood sample were collected in vacutainers. Serum was separated; aliquots were made and stored at -20ºC until further analysis. This study was approved by college ethical review committee.

Body Mass Index Calculation (BMI)

BMI was calculated as kg/m2. Height was measured using a Harpendenanthropometer (Holtain, Ltd, Crymych, UK) to the nearest centimeter and weight was measured using a Scale-tronix scale (Sharp Corp, Wheaton, IL, model 695,weighing to 364 kg). All the subjects were divided into 3 grades i.e. grade 1, grade 2 and grade 3, depending on a corresponding body mass index (BMI) of > 25-29.9, > 30-39.9 and > 40 kg/m2 respectively (FranckeS et al 1997) according to the World Health Organization obesity chart recommendation.

Estimation of Lipid Profile

Lipid profile was assessed by kit method, total serum cholesterol (TC), triglycerides (TG) and lipoproteins; heavy density lipoproteins (HDL-C) and low density lipoproteins (LDL-C) levels were estimated in the study subjects. The absorbance in each case was measured with automated analyzer from Cobas Integra using kit method as per the instructions of the manufacturer. All samples were processed in a batch at one time to avoid artifacts and variations.

Estimation of Serum Leptin Levels

Circulating serum leptin levels were measured by sandwich ELISA, using Leptin ELISA Kits manufactured by Pro assay (USA). The intra-assay and inter-assay coefficients of variation in case of leptin were 3.6 and 5.2%, respectively. All samples were processed in a batch at one time to avoid artifacts and variations.

Estimation of Leptin Resistance

For use as an index of leptin resistance, we measure the ratio of serum leptin to BMI (LEP/BMI) among the study subjects as reported earlier (Leea, J.H 2001).

PCR Amplification of MC4R Gene

PCR amplification was performed usingcustom design gene specific primers for MC4R gene. Genomic DNA was isolated from the buffy coat of whole bloodfrom study subjects using Qiagenminiprepkit (Qiagen, USA). Two pair of MC4R gene amplifying 302bp and 190 bp of the coding region was used for the study.MC4Rforward (5′-CTGATGGAGGGTGCTACGAGCAAC -3′) and MC4R reverse (5′- GGATGCAAGCAAGGAGCTACA -3′) primer and MC4R Forward (5’ AAGAACAAGAATCTGCATTCACCCA-3’) and MC4R Reverse (GGATGCAAGCAAGGAGCTACA-3’)was used and the reaction was carried out under standard conditions, with 35 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 60 seconds (Farooqi IS et al 2002).Reactions were carried out in a total volume of 20 uL and the amplicons were visualized on 1% agarose gels.

Statistical Analysis

Data was maintained on excel spread sheet. Statistical analysis was performed using statistical package for social sciences (SPSS Inc., Chicago, IL, USA) version 17. Results were presented as mean ± standard deviation. The differences between the non-obese and the obese subjects were analyzedwith student-test'.Pearson's correlation was computed to observe the correlation of leptin with lipid profile.

RESULTS

Percent prevalence of Obesity among the study subjects

A total of 113 subjects whose BMI was calculated ≥ 25 were enrolled in the study as obese subjects. Concurrently, subjects with average BMI of <18 were considered as underweight and were excluded from the study. Percent prevalence of obesity among the study subjects was calculated by measuring body mass index was observed to be 34.90%.

Table 1: Percent Prevalence of Obesity and Dyslipidemia among the Study Subjects

Subject Category	% Prevalence
Normal	52%
UnderNormal Weight	14%
Obese	34.90%
Dyslipidemia	35.63%

Evaluation of serum leptin levels among the study subjects

To evaluate the role of leptin in obesity, we estimated the concentration of circulating serum leptin among the study subjects. The mean average cut off value was calculated as 11.98±1.5ng/ml. Serum leptin concentration is elevated in obese subjects (14.41ng/ml ± 1.19ng/ml) as compared to the normal subjects where the concentration was estimated as 10.49ng/ml±2.48ng/ml (Figure 1).

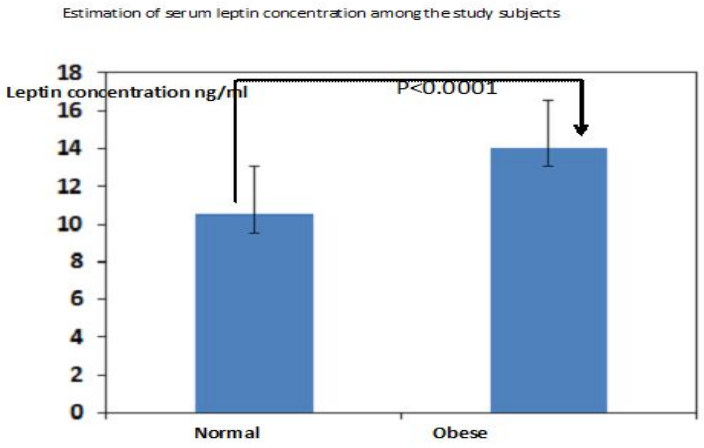

Figure1: Mean Serum Leptin Concentration is Observed to be Significantly Higher Among the Obese Group as Compared to Normal Subjects. TheReference Limit or Cut off Values for Serum Leptin Concentration is Determinedto be 11.98ng/ml.Two Tailed p Values Were Obtained through Student's t Test with a 95% Power (Level of Significance) and a Minimally Significant p Value of 0.0001

Prevalence of Leptin Resistance among the Study Subjects

Since elevated levels of serum leptin was recorded in obese and normal subjects, we sub analyze the serum leptin levels to evaluate the percent prevalence of leptin resistance. It is estimated as 36.53% in obese and 8.3 % in normal subjects (Figure 2). Hence extreme obesity (BMI40) predicted leptin resistance (LEP/BMI90th percentile) with odds ratio of 4.21 (CI=3.01-5.10).

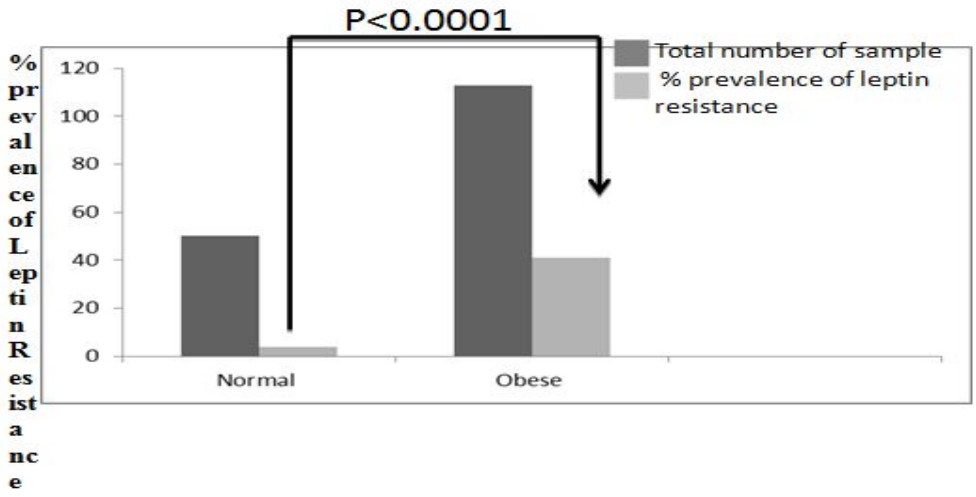

Figure 2: Leptin Resistance is Significantly Higher in Obese Group as Compared to Normal Subjects (P Value is Less than 0.0001). Prevalence of Leptin Resistance among the Study Subjects is expressedas Mean Percent. The Reference Limit or Cut off Values for Serum Leptin Concentration is determinedtobe 11.98ng/ml.

Association of lipid profile with body mass index (BMI): Since anthropometric parameter and lipid profile is an important predictor of obesity. We extrapolate the levels of triglycerides, cholesterol, HDL and LDL with the subjects showing elevated BMI (≥25). Our results shows serum levels of HDL were lower in obese subjects compared to non-obese subjects, P<0.05.Similarly, significantly higher levels of LDL and total cholesterol levels were observed in obese subjects as compared to non-obese subjects, P values being 0.001 (Figure 3).

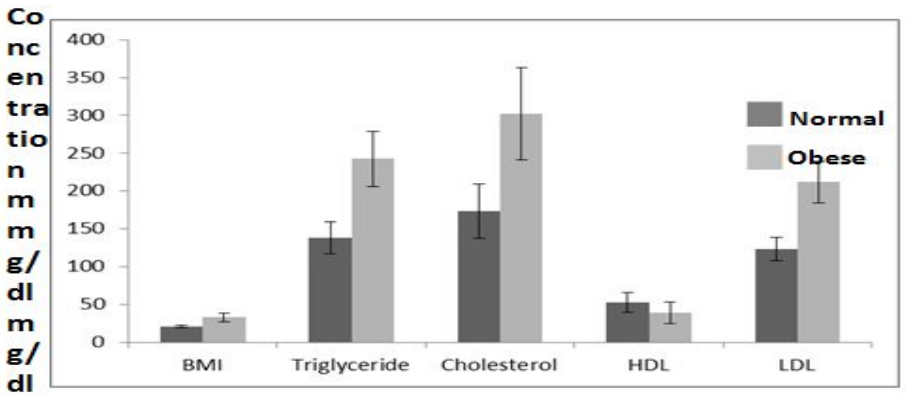

Figure 3: Association of Lipid Profile with Body Mass Index of Study Subjects. Concentration of Triglycerides, Cholesterol, HDL and LDL areMeasured and Compared among the Normal and Obese Subjects. Their Serum Levels Are Expressed as mg/dl and Represented as Mean±SE

Melanocortin 4 Receptor (MC4R) Genotyping: Given that MC4R gene is strongly associated with obesity we evaluated the distribution of MC4R gene among the obese and non-obese Saudi population. MC4R gene was amplified consistently in the normal subjects as compared to obese subjects indicating the possibility of mutation in the obese subjects. To ascertain any major addition or deletions in MC4R gene of obese subjects we amplified the coding region of MC4R gene using two sets of primer. Out of 113 obese subjects 63% shown amplification with the first set of primer (Figure 4A) while 20% of them was amplified with second set of primer. Surprisingly, we didn't get any amplification in the remaining 17% of the obese subjects suggesting the possibility of mutations in the gene. (Figure 4 B).

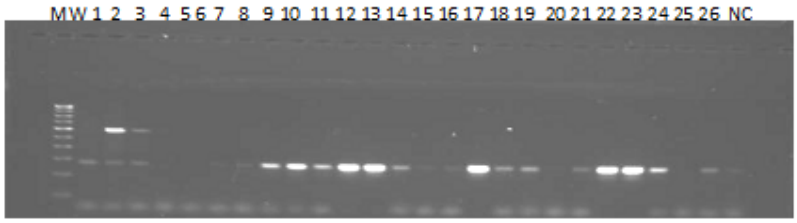

Figure 4A: PCR Amplification of MC4R Gene Using First set of Primer in the Obese Subjects. MW is 100 Base Pair molecular Weight Marker While NC is Negative Control

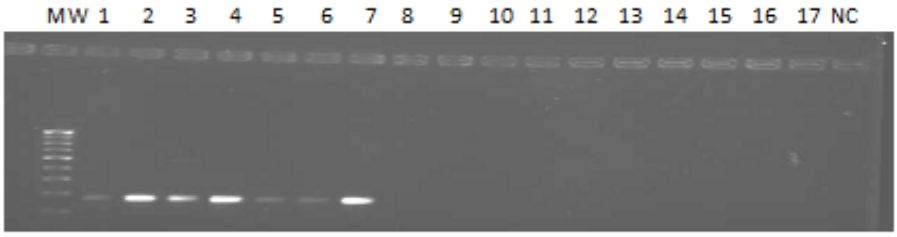

Figure 4B: PCR Amplification of MC4R Gene using Second set of Primer in the Obese Subjects. MW is 100 Base Pair Molecular Weight Marker While NC is Negative Control

DISCUSSIONS

This study evaluated the association of leptin and MC4R gene polymorphism among Saudi obese and non-obesesubjects. Concurrently, measuring the dyslipidemic parameters by extrapolating the prevalence of leptin resistance and biochemical parameters among the subjects.

With respectto serum leptin levels,which is considered as an important regulator of energy homeostatic we observed its levelto behigher in obese than in non-obese subjects, reinforcing the notion that obese have higher leptin levels compared to their normalcounterparts(Figure 1). Given that circulating leptin levelsand leptin resistance are positively co related with obesity we estimated the same between obese and non-obese Saudi subjects. We observed that leptin levels are significantly higher in the obese subjects as compared to non-obese counterparts (Figure 1).This is consistent with the previous reports which also showed that leptin levels are higher among the obese subjects (Farooqi IS et al 2002). When the results were considered according to BMI and leptin resistance (adjusting the circulating leptin levels), it was found that 38.53% are showing leptin resistance as compared to 8.3% in non-obese subjects. This leptin resistance was in agreement with the earlier reports in terms of extreme obesity and leptin resistance (BMI $\geq$ 35, predicted leptin resistance (LEP/BMI90th percentile) with odds ratio of 4.21 (CI=3.01-5.10). (Carter S et al 2013 and Park HK et al 2015).

(Figure 2).Additionally, our results of leptin association with obesity were further positively supported by higher levels of triglyceride (242.42± 36.39mg/dl), cholesterol (302±61.00mg/dl) and LDL cholesterol (212.01±27.31mg/dl) in obese subjects as compared to normal subjects (Figure 3). These results are in in contrast to the study by Rahman Al Nuaim et al 1997 who found that there was no significant difference in the mean serum level of total cholesterol concentration between control and obese groups. However we found significantly lower mean serum HDL concentration among the obese group, which was in agreement with aforementioned study.

Further, we determine the risk of dyslipidemia among the obese subjects by extrapolating the biochemical parameters we studied. Our results suggest that approximately 35.63% of Saudi obese subjects are at risk of developing dyslipidemia (Table-1).Our findings are in agreement with the national survey reports which suggest that the overall prevalence of dyslipidemia is 20- 40 % among Saudi population (Al-Kaabba et al 2012). Aljaberi et al while studyingthe relationshipbetween overweight, obesity and plasma lipids in Saudi adults with Type 2 Diabetes noted that the prevalence of dyslipidemia was 18 -28% among non-diabetic obese subjects.

Having established the positive association of circulating serum leptin levels and leptin resistance among the obese subjects, we studied the central molecular signature of obesity; the MC4R gene (Farooqi IS et al 2007).The MC4R gene although was consistently amplified in all the normal subjects which we studied, but significant variation was observed in obese subjects (Figura4 A and 4B). Although no major addition or deletion was apparently observed but the gene was not consistently amplified indicating the possible presence of mutation. Our results are in accordance with the previous studies which show that mutations in MC4R gene results obesity in 63.5% of European subjects and it also variously effects eating/satiety signaling (Farooqi IS et al 2007). These individuals eat more at a test meal than those who have mutations which partially disrupt signaling. In some cases, the alteration of the basal activity of the receptor (Lubrano-Berthelier C et al 2003 and Yeo GS et al 2003) has also been reported.A more specific type of mutation inMC4R was also reported by Michaela Pichler et al (2008) they genotyped a group of 1029 severely obese white subjects for MC4R mutation. These subjects were having an average body mass index (BMI; in kg/m2) of 46.0 (range: 33–92). They found that the carriers of the mutation103I had significantly higher daily energy (364 kcal/d or 19%; P = 0.03) and carbohydrate (57 g/d or 27%; P = 0.01) intakes than non-carriers.

CONCLUSIONS

Thus our study is one the first report from south western province of Saudi Arabia to demonstrate association of leptin hormone and melanocortin 4 receptor gene among the obese subjects. In future, however a whole genome sequencing of the extremely obese subject, followed by genetic variance analysis will be carried out to get asignificant insight in the causes of obesity. Moreover sequencing of specified translational regions with SNP in functional motifs may yield meaningful insights. Also given to the high rate of consanguinity in KSA it would be interesting to see the transmission dynamics of MC4R and other genetic players of obesity.

ACKNOWLEDGEMENT

The project was funded by Deanship of Scientific Research, Jazan University, Jazan, KSA. We would like to thank all the study subjects including voluntary controls who participated and co-operated in this study.

Conflict of Interest: All authors have declared that there is no conflict of interest

REFERENCES

1. *Aljabri, K.S and Bokhari, S.A et al.(2015). The Relation between Overweight, Obesity and Plasma Lipids in Saudi Adults with Type 2 Diabetes. Int J Diabetes Clin Res, 2:3*
2. *Al-Kaabba, A.F and Al-Hamdan, N.A et al. (2012) Prevalence and Correlates of Dyslipidemia among Adults in Saudi Arabia: Results from a National Survey. Open Journal of Endocrine and Metabolic Diseases, 2, 89-97.*
3. *Baskin, D.G.1, and FiglewiczLattemann D, et at (1999). Insulin and leptin: dual adiposity signals to the brain for the regulation of food intake and body weight. Brain Res. 27;848(1-2):114-23.*
4. *Bochukova, E.G, Huang, N, et al. (2010). Large, rare chromosomal deletions associated with severe early-onset obesity: Nature 4; 463(7281):666-70.*
5. *Carter S, and Caron, A et al (2013). Role of leptin resistance in the development of obesity in older patients. ClinInterv Aging. 8: 829–844. Published online 2013 Jul 4. doi: 10.2147/CIA.S36367.*
6. *Considine, R.V, and Sinha, M.K, et al. (1996). Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med. 334:292–5.*
7. *Dyck, D.J, and Heigenhauser, G.J.F, et al (2006). The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. Acta Physiol. 186:5–16.*
8. *Farooqi, I.S, and Matarese, G et al (2002). Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. J Clin Invest. 110:1093–1103. doi: 10.1172/JCI0215693*
9. *Farooqi, S, and Keogh, E.B.J, et al (2007). Leptin Regulates Striatal Regions and Human Eating Behavior: I. Science 317; 7 - 1355.*
10. *Francke S, and Clement K, et al. (1997). Genetic studies of the leptin receptor gene in morbidly obese French Caucasian families. Hum Genet. 100(5–6):491–496.*
11. *Huszar D, and Lynch, C.A, et al (1997). Targeted disruption of the melanocortin-4 receptor results in obesity in mice. Cell. 88:131–141.*
12. *Hwa, J. J., and Ghibaudi, L., et al (1996). Intracerebroventricular injection of leptin increases thermogenesis and mobilizes fat metabolism in ob/ob mice. Horm. Metab. Res. 28, 659–663.*
13. *Kopelman P.G (2000). Obesity as a medical problem. Nature 404:635– 643.*
14. *Leea, J.H and Reedb, D.R (2001). Leptin resistance is associated with extreme obesity and aggregates in families. Nature 25;10, 1471-1473.*
15. *Lubrano-Berthelier C, and Cavazos M, et al (2003). The human MC4R promoter: characterization and role in obesity. Diabetes 52:2996 –3000.*
16. *Lubrano-Berthelier, C, and Dubern, B, et al (2006). Melanocortin 4 Receptor Mutations in a Large Cohort of Severely Obese Adults: Prevalence, Functional Classification, Genotype-Phenotype Relationship, and Lack of Association with Binge Eating. J ClinEndocrinolMetab, 91(5):1811–1818.*
17. *Mizuno,T.M, Makimura, H, et al (1999). Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. Endocrinology. 140:4551–4557.*

18. *Montague, C.T, and Farooqi, I.S, et al.(2001). Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387(6636):903–8.*
19. *Oswal A, and Yeo, G.S. (2007). The leptin melanocortin pathway and the control of body weight: lessons from human and murine genetics. Obes Rev. 8:293–306*
20. *Park H.K and Ahima R.S (2015). Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism. 64(1):24-34. doi: 10.1016/j.metabol.2014.08.004. Epub 2014 Aug 15.*
21. *Pichler, M, and Kollerits, B et al (2008). Association of the melanocortin-4 receptor V103I polymorphism with dietary intake in severely obese persons. Am J ClinNutr; 88:797–800.*
22. *Pichler, M, and Kollerits, B, et al (2008). Association of the melanocortin-4 receptor V103I polymorphism with dietary intake in severely obese persons. Am J ClinNutr; 88:797–800.*
23. *Prentice A.M (2006). The emerging epidemic of obesity in developing countries. Int J Epidemiol 35:93–99.*
24. *Rahman Al-Nuaim A1. (1997). Effect of overweight and obesity on glucose intolerance and dyslipidemia in Saudi Arabia, epidemiological study. Diabetes Res ClinPract. 36(3):181-91.*
25. *Seidell J.C (1997). Time trends in obesity: an epidemiological perspective. HormMetab Res 29:155–158.*
26. *Vaisse, C.I, and Halaas, J.L et al (1996). Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. Nat.Genet 14(1):95-7.*
27. *Wolf A.M and Colditz G.A (1998). Current estimates of the economic cost of obesity in the United States. Obes Res 6:97–106*
28. *Yeo G.S, and Lank, E.J, et al (2003): Mutations in the human melanocortin-4 receptor gene associated with severe familial obesity disrupts receptor function through multiple molecular mechanisms. Hum Mol Genet 12:561–574.*